WHAT IS CLAIMED IS:

1. Small hepatocyte-rich colonies, in which at least 70 % of the total cells constituting each of the colonies are the small hepatocytes.

2. The small hepatocyte-rich colonies according to claim 1, wherein the total number of cells constituting each colony is 10 to 30.

3. A process for preparing small hepatocyte-rich colonies which comprises the steps of:

(i) isolating hepatocytes from a liver,

(ii) dividing the isolated hepatocytes into a heavy fraction enriched with parenchymal cells and a light fraction enriched with non-parenchymal cells but having a low parenchymal cell content, and recovering the light fraction,

(iii) culturing the cells in the light fraction in a culture medium containing nicotinamide to form small hepatocyte colonies, and

(iv) recovering the small hepatocyte colonies having a total number of cells of 10 to 30.

4. A process for preparing small hepatocyte-rich colonies, which comprises the steps of:

(i) dissociating colonies from a culture dish by reacting an enzyme or without reacting an enzyme on small hepatocytes forming the colonies to recover the small hepatocytes,

(ii) successively subculturing the recovered small hepatocytes by using a culture medium containing nicotinamide to form colonies each composed of 10 to 30 cells in total, and

(iii) recovering the colonies, each composed of 10 to 30 cells in total.

5. A process for maturating small hepatocyte-rich colonies into a liver tissue, which comprises the steps of:

(i) culturing the small hepatocyte-rich colonies according to claim 1,

(ii) adding an extracellular matrix to the medium containing the cultured small hepatocyte-rich colonies and

(iii) further culturing the small hepatocyte-rich colonies, which have been cultured in

the extracellular matrix-containing medium, in an extracellular matrix-free medium.

6. The process for maturating small hepatocyte-rich colonies into a liver tissue according to claim 5, wherein the density of the small hepatocyte-rich colonies is 200 to 1,000 colonies/cm^2 at the initial stage of the culture.

7. A process for maturating small hepatocyte-rich colonies into a liver tissue, which comprises the steps of:

(i) placing the small hepatocyte-rich colonies according to claim 1 on a bioabsorbable sheet, and

(ii) placing the sheet in a culture medium to culture the small hepatocyte-rich colonies on the sheet.

8. The maturation process according to claim 7, wherein the density of the colonies on the sheet is 200 to 1,000 colonies/cm^2 at the initial stage of the culture.

9. A process for preparing a liver tissue for transplantation, which comprises the steps of:

(i) placing the small hepatocyte-rich colonies according to claim 1 on a bioabsorbable sheet, and

(ii) placing the sheet in a serum-free culture medium to culture the small hepatocyte-rich colonies on the sheet.

10. The process for preparing the liver tissue for transplantation according to claim 9, wherein the density of the colonies on the sheet is 200 to 1,000 colonies/cm^2 at the initial stage of the culture.

11. A method of estimating an effect of a chemical substance on a liver function *in vitro*, which comprises the steps of contacting the small hepatocyte-rich colonies matured according to the process of claim 5 with the chemical substance, identifying a gene the expression of which is

induced or repressed in the cells in the small hepatocyte-rich colonies, and/or determining the expression level thereof.

12. A method of estimating an effect of a chemical substance on a liver function *in vitro*, which comprises the steps of contacting the small hepatocyte-rich colonies matured according to the process of claim 7 with the chemical substance, identifying a gene the expression of which is induced or repressed in the cells in the small hepatocyte-rich colonies, and/or determining the expression level thereof.

13. A method of estimating an effect of a chemical substance on a liver function *in vitro*, which comprises the steps of contacting the small hepatocyte-rich colonies matured according to the process of claim 5 with a chemical substance, identifying a gene the expression of which is induced or repressed in the cells in the small hepatocyte-rich colonies, and/or determining the expression level thereof, comparing the induction or repression pattern of the gene the expression of which is induced or repressed with an induction or repression pattern of the gene associated with a chemical substance having a known effect, and estimating the effect of the chemical substance on the liver function *in vitro* which exhibits the induction or repression pattern similar to the pattern of the chemical substance having a known effect as the effect of the chemical substance placed in contact with the small hepatocyte-rich colonies.

14. A method of estimating an effect of a chemical substance on a liver function *in vitro*, which comprises the steps of contacting the small hepatocyte-rich colonies matured according to the process of claim 7 with a chemical substance, identifying a gene the expression of which is induced or repressed in the cells in the small hepatocyte-rich colonies, and/or determining the expression level thereof, comparing the induction or repression pattern of the gene the expression of which is induced or repressed with an induction or repression pattern of the gene associated with a chemical substance having a known effect, and estimating the effect of the chemical substance on the liver function *in vitro* which exhibits the induction or repression pattern similar to the pattern of the chemical substance having a known effect as the effect of the chemical

substance placed in contact with the small hepatocyte-rich colonies.

15. The method according to claim 11, wherein the gene the expression of which is induced or repressed is a drug-metabolizing enzyme gene.

16. The method according to claim 12, wherein the gene the expression of which is induced or repressed is a drug-metabolizing enzyme gene.

17. The method according to claim 13, wherein the gene the expression of which is induced or repressed is a drug-metabolizing enzyme gene.

18. The method according to claim 14, wherein the gene the expression of which is induced or repressed is a drug-metabolizing enzyme gene.

19. A method for determining whether a chemical substance induces or represses the expression of a drug-metabolizing enzyme in the liver, which comprises contacting the small hepatocyte-rich colonies matured by the process according to claim 5 with a chemical substance and determining a capacity of the chemical substance to induce or repress the expression of the drug-metabolizing enzyme gene in the cells in the small hepatocyte-rich colonies.

20. A method for determining whether a chemical substance induces or represses the expression of a drug-metabolizing enzyme in the liver, which comprises contacting the small hepatocyte-rich colonies matured by the process according to claim 7 with a chemical substance and determining a capacity of the chemical substance to induce or repress the expression of the drug-metabolizing enzyme gene in the cells in the small hepatocyte-rich colonies.

21. The method according to claim 15, wherein the drug-metabolizing enzyme gene is the gene encoding cytochrome P450 enzyme.

22. The method according to claim 16, wherein the drug-metabolizing enzyme gene is the gene encoding cytochrome P450 enzyme.

23. The method according to claim 17, wherein the drug-metabolizing enzyme gene is the gene encoding cytochrome P450 enzyme.

24. The method according to claim 18, wherein the drug-metabolizing enzyme gene is the gene encoding cytochrome P450 enzyme.

25. The method according to claim 19, wherein the drug-metabolizing enzyme gene is the gene encoding cytochrome P450 enzyme.

26. The method according to claim 20, wherein the drug-metabolizing enzyme gene is the gene encoding cytochrome P450 enzyme.

27. The method according to claim 21, wherein the drug-metabolizing enzyme gene is selected from the group consisting of the enzymes encoding CYP2B1, CYP3A2, CYP2E1 and CYP4A1.

28. The method according to claim 22, wherein the drug-metabolizing enzyme gene is selected from the group consisting of the enzymes encoding CYP2B1, CYP3A2, CYP2E1 and CYP4A1.

29. The method according to claim 23, wherein the drug-metabolizing enzyme gene is selected from the group consisting of the enzymes encoding CYP2B1, CYP3A2, CYP2E1 and CYP4A1.

30. The method according to claim 24, wherein the drug-metabolizing enzyme gene is selected from the group consisting of the enzymes encoding CYP2B1, CYP3A2, CYP2E1 and

CYP4A1.

31. The method according to claim 25, wherein the drug-metabolizing enzyme gene is selected from the group consisting of the enzymes encoding CYP2B1, CYP3A2, CYP2E1 and CYP4A1.

32. The method according to claim 26, wherein the drug-metabolizing enzyme gene is selected from the group consisting of the enzymes encoding CYP2B1, CYP3A2, CYP2E1 and CYP4A1.

33. A method of estimating the effect of a chemical substance connected with the liver function *in vitro*, which comprises the steps of placing the small hepatocyte-rich colonies matured according to the process of claim 5 together with a chemical substance, identifying a gene the expression of which is induced or repressed in the cells in the above-described small hepatocyte-rich colonies, and/or determining the expression level thereof.

34. A method of estimating the effect of a chemical substance connected with the liver function *in vitro*, which comprises the steps of placing the small hepatocyte-rich colonies matured according to the process of claim 7 together with a chemical substance, identifying a gene the expression of which is induced or repressed in the cells in the above-described small hepatocyte-rich colonies, and/or determining the expression level thereof.